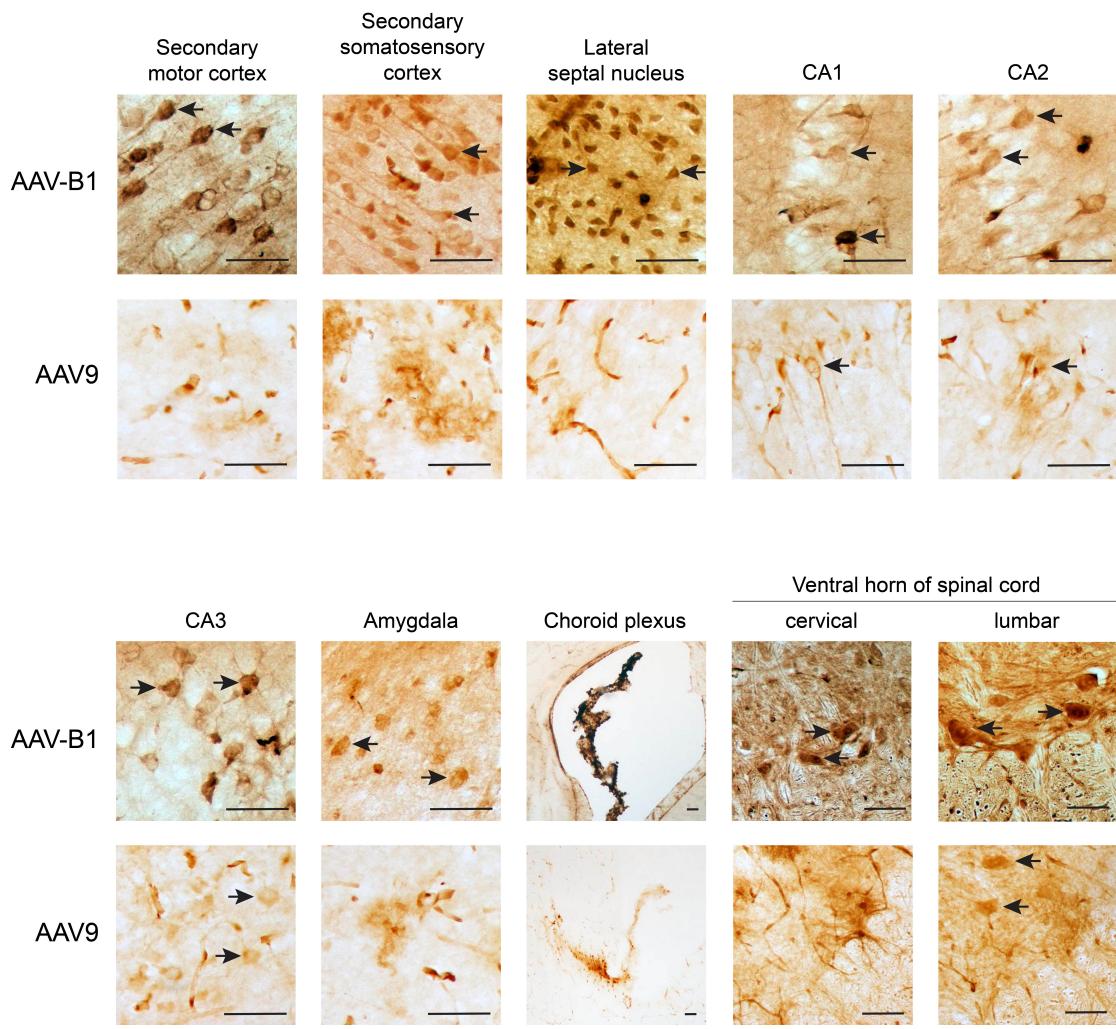
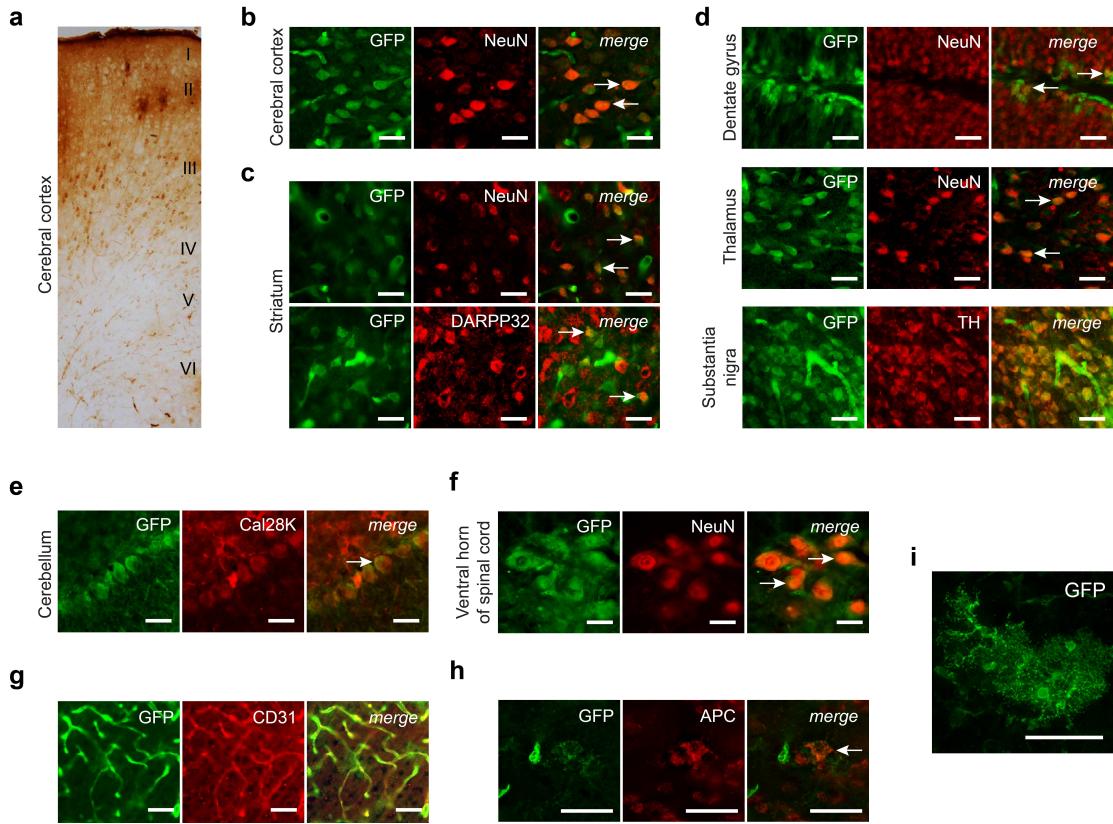


**Supplementary Figure S1: Chimeric nature of packaged viral library.** **a.** Parental capsid contribution to five capsid genes PCR amplified from packaged viral library. **b.** Homology between capsids at the amino acid level. Grey areas indicate homology; black lines indicate non-homologous amino acids. % homology is calculated for amino acid composition.



**Supplementary Figure S2: Transduction profile of AAV-B1 and AAV9 vector across multiple CNS regions after systemic delivery.** Black arrows indicate examples of GFP-positive neurons. Bar = 50  $\mu$ m.



**Supplementary Figure S3: Phenotype of GFP positive cells in CNS after systemic delivery of AAV-B1.** (a) Chromogenic GFP staining in layers I-VI of cerebral cortex. (b-h) Transduced cells were identified by double immunofluorescence staining with antibodies to GFP, pan-neuronal marker NeuN (b, c, d and f), striatal medium spiny neuron marker DARPP32 (c), dopaminergic neuron marker tyrosine hydroxylase (TH) (d), Purkinje neuron marker calbindin-D-28k (Cal28K) (e), endothelial marker CD31 (g), and mature oligodendrocyte marker APC (h). The large size, morphology and location of GFP-positive neurons in the ventral spinal cord suggest a motor neuron identity. GFP-positive astrocytes (i) were identified based on their morphology. White arrows indicate examples of co-localization. Bar = 10  $\mu$ m.

## VP1



AAV8	1	MAADGYLPDWLEDNLSEGIREWWALKPGAPPKPANQQKQDDGRGLVLPGYKYLGPFGNLDKGEPVNAADAAALEHDKAYD	80
AAV-B1	1	MAADGYLPDWLEDNLSEGIREWWDLKPGAPPKPANQQKQDDGRGLVLPGYKYLGPFGNLDKGEPVNAADAAALEHDKAYD	80
AAV1	1	MAADGYLPDWLEDNLSEGIREWWDLKPGAPPKPANQQKQDDGRGLVLPGYKYLGPFGNLDKGEPVNAADAAALEHDKAYD	80
AAV2	1	MAADGYLPDWLEDNLSEGIREWWDLKPGAPPKPANQQKQDDGRGLVLPGYKYLGPFGNLDKGEPVNEADAAALEHDKAYD	80
AAV5	1	MSFVDHPDPWLEE-VCEGLREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPNGNLDKGEPVNRADAREHDISYN	79
AAV6	1	MAADGYLPDWLEDNLSEGIREWWDLKPGAPPKPANQQKQDDGRGLVLPGYKYLGPFGNLDKGEPVNAADAAALEHDKAYD	80
AAV7	1	MAADGYLPDWLEDNLSEGIREWWDLKPGAPPKPANQQKQDNNGRGLVLPGYKYLGPFGNLDKGEPVNAADAAALEHDKAYD	80
AAV9	1	MAADGYLPDWLEDNLSEGIREWWALKPGAPPKPANQQHQDNARGLVLPGYKYLGPFGNLDKGEPVNAADAAALEHDKAYD	80
AAVrh10	1	MAADGYLPDWLEDNLSEGIREWWDLKPGAPPKPANQQKQDDGRGLVLPGYKYLGPFGNLDKGEPVNAADAAALEHDKAYD	80

## VP2



AAV8	81	QQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSQRSQPDSTSSTGI	160
AAV-B1	81	QQIKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP <span style="background-color: red;">F</span> GLVEEGAKTAPGKKRPVE <span style="background-color: red;">QSPQE</span> -PDSSSGI	159
AAV1	81	QQIKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVE <span style="background-color: red;">QSPQE</span> -PDSSSGI	159
AAV2	81	RQLDSGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAGKRPVEHSPV <span style="background-color: red;">E</span> -PDSSSGT	159
AAV5	80	EQLEAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP <span style="background-color: red;">F</span> GLVEEGAKTAPGKRIDDHF <small>P</small> KR-KKART--	156
AAV6	81	QQIKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEP <span style="background-color: red;">F</span> GLVEEGAKTAPGKKRPVE <span style="background-color: red;">QSPQE</span> -PDSSSGI	159
AAV7	81	QQIKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPAKKRVEPSQRSQPDSTSSTGI	160
AAV9	81	QQIKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVE <span style="background-color: red;">QSPQE</span> -PDSSAGI	159
AAVrh10	81	QQIKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVE <span style="background-color: red;">QSPQE</span> -PDSSAGI	160

## VP3



AAV8	161	GKKGQQPARKRLNFGQTGDSEVPDPQPLGEPPAAPSG-VGPNTMAAGGGAPMADNNNEGADGVGSSSGNWHCDSTWLGDR	239
AAV-B1	160	GKKGQQPARKRLNFGQTGDSEVPDPQPLGEPPAAPSG-VGPNTMAAGGGAPMADNNNEGADGVG <span style="background-color: red;">N</span> SSGNWHCDSTWL <span style="background-color: red;">M</span> GDR	238
AAV1	160	GKTGQQPAKKRLNFGQTGDSEVPDPQPLGEPPATPAA-VGPTTMASGGGAPMADNNNEGADGVG <span style="background-color: red;">N</span> ASGNWHCDSTWLGDR	238
AAV2	160	GKAGQQPARKRLNFGQTGDADSVDPDPQPLGQPPAAPSG-LGTNTMATGSGAPMADNNNEGADGVG <span style="background-color: red;">N</span> SSGNWHCDSTWL <span style="background-color: red;">M</span> GDR	238
AAV5	157	----EEDSKP---STSSDAEAGPSGSQQLQIPAQPASSLGA <span style="background-color: red;">D</span> TMSAGGGPLGDN <span style="background-color: red;">N</span> QGADGVG <span style="background-color: red;">N</span> ASGDWHCDSTWL <span style="background-color: red;">M</span> GDR	228
AAV6	160	GKTGQQPAKKRLNFGQTGDSEVPDPQPLGEPPATPAA-VGPTTMASGGGAPMADNNNEGADGVG <span style="background-color: red;">N</span> ASGNWHCDSTWLGDR	238
AAV7	161	GKKGQQPARKRLNFGQTGDSEVPDPQPLGEPPAAPSS-VGSGTVAAAGGGAPMADNNNEGADGVG <span style="background-color: red;">N</span> ASGNWHCDSTWLGDR	239
AAV9	160	GKSGAQPAKKRLNFGQTGDTEVPDPQPIGEPPAAPSG-VGSLTMASGGGAPVADNNNEGADGVGSSSGNWHCDSQWLGDR	238
AAVrh10	161	GKKGQQPAKKRLNFGQTGDSEVPDPQPIGEPPAGPSG-LGSGTMAAGGGAPMADNNNEGADGVGSSSGNWHCDSTWLGDR	239

		$\beta B$	I	$\beta C$	$\alpha 1$	$\beta D$	
AAV8	240	VITTSTRTWALPTYNHNLKYKQISNGTSGGATNDNTYFGYSTPWGYFDFNRFCHFSPRDWQRLINNNWGFRPKRLSFKLF					319
AAV-B1	239	VITTSTRTWALPTYNHNLKYKQISNGTSGG <span style="background-color: red;">ST</span> NDNTYFGYSTPWGYFDFNRFCHFSPRDWQRLINNNWGFRPKRLSFKLF					318
AAV1	239	VITTSTRTWALPTYNHNLKYKQISSASTG-ASNDNHYFGYSTPWGYFDFNRFCHFSPRDWQRLINNNWGFRPKRLNFKLF					317
AAV2	239	VITTSTRTWALPTYNHNLKYKQISSQS-G-ASNDNHYFGYSTPWGYFDFNRFCHFSPRDWQRLINNNWGFRPKRLNFKLF					316
AAV5	229	VVTKSTRTRWVLPSYNHHYREIKSGSVD-GSNANAYFGYSTPWGYFDFNRFCHFSPRDWQRLINNNYWGFRPRSLRVKIF					307
AAV6	239	VITTSTRTWALPTYNHNLKYKQISSASTG-ASNDNHYFGYSTPWGYFDFNRFCHFSPRDWQRLINNNWGFRPKRLNFKLF					317
AAV7	240	VITTSTRTWALPTYNHNLKYKQISSETA-C <span style="background-color: red;">ST</span> NDNTYFGYSTPWGYFDFNRFCHFSPRDWQRLINNNWGFRPKRLRFKLF					318
AAV9	239	VITTSTRTWALPTYNHNLKYKQISNSTSGGSNDNAYFGYSTPWGYFDFNRFCHFSPRDWQRLINNNWGFRPKRLNFKLF					318
AAVrh10	240	VITTSTRTWALPTYNHNLKYKQISNGTSGG <span style="background-color: red;">ST</span> NDNTYFGYSTPWGYFDFNRFCHFSPRDWQRLINNNWGFRPKRLNFKLF					319
		II	$\beta E$		III		
AAV8	320	NIQVKEVTQNEGKTIAANLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMI <span style="background-color: red;">P</span> QYGYLTLN--NGSQAVGRSSFYC					397
AAV-B1	319	NIQVKEVTQNEGKTIAANLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMI <span style="background-color: red;">P</span> QYGYLTLN--NGSQAVGRSSFYC					396
AAV1	318	NIQVKEVTNDGVTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMI <span style="background-color: red;">P</span> QYGYLTLN--NGSQAVGRSSFYC					395
AAV2	317	NIQVKEVTNDGVTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMI <span style="background-color: red;">P</span> QYGYLTLN--NGSQAVGRSSFYC					394
AAV5	308	NIQVKEVTQDSTTTIANNLTSTVQVFTDDYQLPYVVGNGETGCLPAFPQQVFTLPQYGYATLNRDNTENPTERSSFFC					387
AAV6	318	NIQVKEVTNDGVTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMI <span style="background-color: red;">P</span> QYGYLTLN--NGSQAVGRSSFYC					395
AAV7	319	NIQVKEVTNDGVTTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMI <span style="background-color: red;">P</span> QYGYLTLN--NGSQSVGRSSFYC					396
AAV9	319	NIQVKEVDNNNGVKTIANNLTSTVQVFTDSYQLPYVLGSAHEGCLPPFPADVFMI <span style="background-color: red;">P</span> QYGYLTLN--DGSQAVGRSSFYC					396
AAVrh10	320	NIQVKEVTQNEGKTIAANLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMI <span style="background-color: red;">P</span> QYGYLTLN--NGSQAVGRSSFYC					397
		$\beta F$	$\beta G$		IV		
AAV8	398	LEYFPSQMLRTGNNFQFTYTFEDVPFHSSYAHQSLSLDRLMNPLIDQYLYYLTSRTQTT-CGTANTQTLGFSQGGPNTMANQ					476
AAV-B1	397	LEYFFPSQMLRTGNNF <span style="background-color: red;">E</span> FSYQFEDVPFHSSYAHQSLSLDRLMNPLIDQYLYYLTSRTQ <span style="background-color: red;">S</span> T-CGTARTQQL <span style="background-color: red;">L</span> FSQAGPNTMANQ					475
AAV1	396	LEYFPSQMLRTGNNFTFSYT <span style="background-color: red;">T</span> FEVVPFHSSYAHQSLSLDRLMNPLIDQYLYYLTSRTQ <span style="background-color: red;">S</span> T-QGAQN <span style="background-color: red;">K</span> DLLFSRGSPAGMSVQ					474
AAV2	395	LEYFPSQMLRTGNNFTFSYT <span style="background-color: red;">T</span> FEDVPFHSSYAHQSLSLDRLMNPLIDQYLYYLTSRTNTP-SGTTTQSRLQFSQAGASDIRDQ					473
AAV5	388	LEYFPSKMLRTGNNFTFSYT <span style="background-color: red;">T</span> YNEEVVPFHSSFAPSONLFKLANPLVDQYLYRFVSTNNT-GGVQFNKNL-----AGR <span style="background-color: red;">Y</span> AN					459
AAV6	396	LEYFPSQMLRTGNNFTFSYT <span style="background-color: red;">T</span> FEDVPFHSSYAHQSLSLDRLMNPLIDQYLYYLTSRTQ <span style="background-color: red;">N</span> Q-SGAQN <span style="background-color: red;">K</span> DLLFSRGSPAGMSVQ					474
AAV7	397	LEYFPSQMLRTGNNFQFSYS <span style="background-color: red;">T</span> EVENVPFHSSYAHQSLSLDRLMNPLIDQYLYYLARTQSNPGGTAGNRELQFYQGGPSTMAEQ					476
AAV9	397	LEYFPSQMLRTGNNF <span style="background-color: red;">E</span> FSYQFEDVPFHSSYAHQSLSLDRLMNPLIDQYLYYLTSK-T-IN-GSGQNQQT <span style="background-color: red;">L</span> KFSVAGPSNMAVQ					474
AAVrh10	398	LEYFPSQMLRTGNNF <span style="background-color: red;">E</span> FSYQFEDVPFHSSYAHQSLSLDRLMNPLIDQYLYYLTSRTQ <span style="background-color: red;">S</span> T-CGTAGTQQL <span style="background-color: red;">L</span> FSQAGPNMSAQ					476

		V	VI	VII	
AAV8	477	A-KNWLPGPCYRQQRVSTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFGKQNAARDNA			555
AAV-B1	476	A-KNWLPGPCYRQQRVSTTGQNNNSNSAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFGKQNAARDNA			554
AAV1	475	P-KNWLPGPCYRQQRVSKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDEDKFFFPMMSGVMIFGKESAGASNT			553
AAV2	474	S-RNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFPQSGVLIFGKQGSEKTNV			552
AAV5	460	TYKNWFPGPGRTOGWNLGSGVNRASVSAFATTNRMELEGASYQVFPQPNGMNTNLQGSNTYALENTMIFNSQPANPGTT			539
AAV6	475	P-KNWLPGPCYRQQRVSKTDNNNSNFTWTGASKYNLNGRESIINPGTAMASHKDDEKFFFPMMSGVMIFGKESAGASNT			553
AAV7	477	A-KNWLPGPCFRQQRVSKLDQNNNSNFAWTGATKYHLNGRNSLVNPGVAMATHKDDEDRFFPSSGVLFGKTGA-TNKT			554
AAV9	475	G-RNYIPGPSYRQQRVSTVTQNNSNEFAWPGASWALNGRNSLMPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNV			553
AAVrh10	477	A-KNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPGVAMATHKDDEERFFPSSGVLMFGKQGAGKDNV			555

### VIII

AAV8	556	DY---SDVMLTSEEETKTTNPVATEEYGIVADNLQQONTAPQIGTVNSQGALPGMVWQRDVYLQGPIWAKIPHTDGNFH	632
AAV-B1	555	DY---SDVMLTSEEETKTTNPVATEEYGIVADNLQQONTAPQIGTVNSQGALPGMVWQRDVYLQGPIWAKIPHTDGNFH	631
AAV1	554	AL---DNVMITDEEEIKATNPVATERFGTVAVNFQSSSTDPATGDVHAMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFH	630
AAV2	553	DI---EKVMITDEEEIRTNPVATEEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFH	629
AAV5	540	ATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQOEIVPGSVWMERDVYLQGPIWAKIPHTGAHFH	619
AAV6	554	AL---DNVMITDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQGPIWAKIPHTDGHFH	630
AAV7	555	TL---ENVLMTNEEEIRPTNPVATEEYGIVSSNLQAANTAAQTQVNNNQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFH	631
AAV9	554	DA---DKVMITNEEEEIKTTNPVATESYGOVATNHOSAQAOAQATGVWQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFH	630
AAVrh10	631	DY---SSVMLTSEEETKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFH	632

$\beta_H$

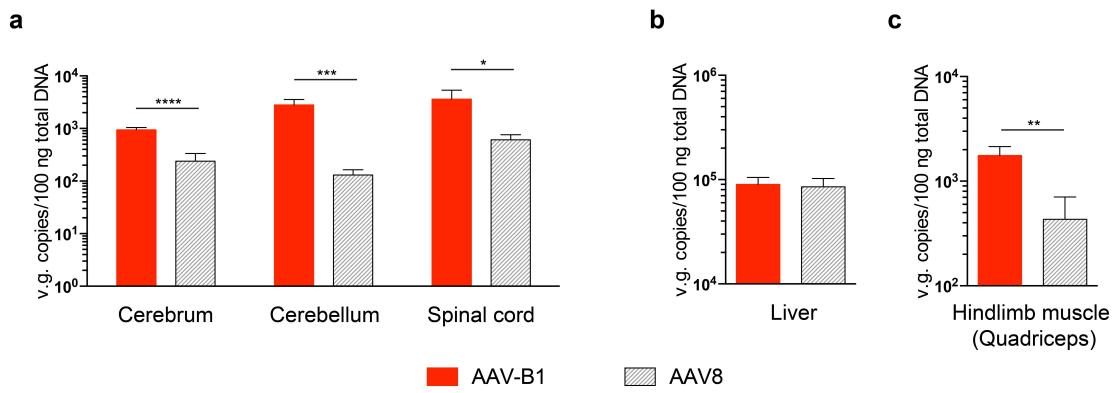
$\beta_I$

IX

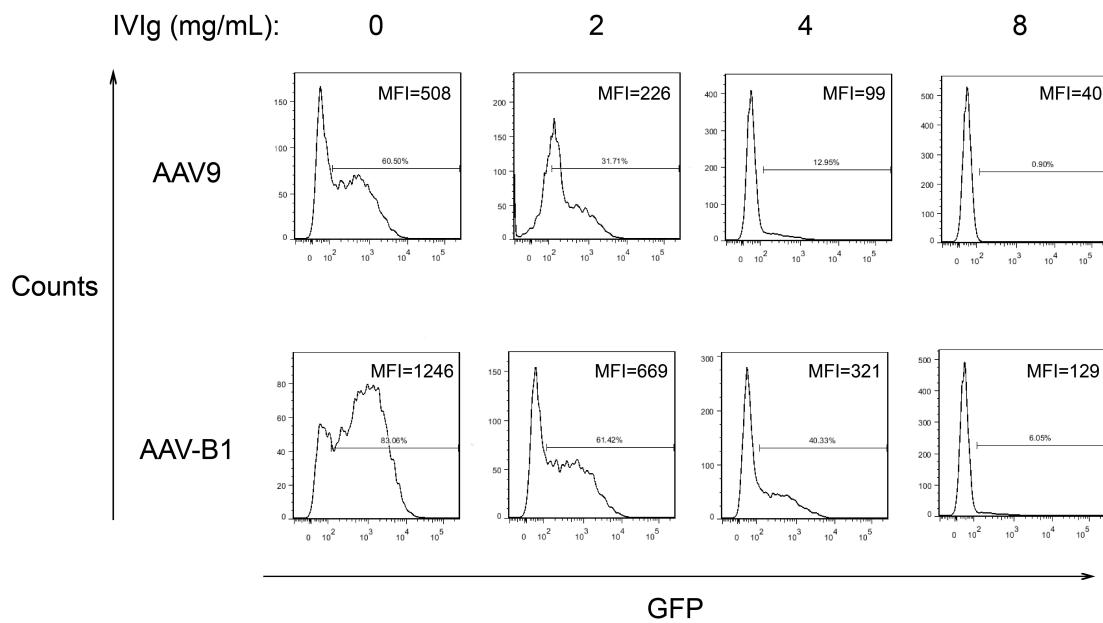
AAV8	633	PSPLMGGFGLKHPPPQIILIKNTPVPADPPTTFNOSKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTS	712
AAV-B1	632	PSPLMGGFGLKHPPPQIILIKNTPVPADPPTTFNOSKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTS	711
AAV1	631	PSPLMGGFGLKNPPPQIILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEVQYTSNYYAKSAN	710
AAV2	630	PSPLMGGFGLKHPPPQIILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIYQYTSNYYKSVN	709
AAV5	620	PSPAMGGFGLKHPPPQIILIKNTPVPNPGN-ITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNYYNDPQF	698
AAV6	631	PSPLMGGFGLKHPPPQIILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEVQYTSNYYAKSAN	710
AAV7	632	PSPLMGGFGLKHPPPQIILIKNTPVPANPPEVTPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIYQYTSNFEKQTG	711
AAV9	631	PSPLMGGFGMKHPPPQIILIKNTPVPADPPTAFNDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNN	710
AAVrh10	633	PSPLMGGFGLKHPPPQIILIKNTPVPADPPTFSQAKLASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTN	712

AAV8	713	VDFAVNTEGVYSEPRPIGTRYLTRNL	738
AAV-B1	712	VDFAVNTEGVYSEPRPIGTRYLTRNL	737
AAV1	711	VDFTVDNNGLYTEPRPIGTRYLTRPL	736
AAV2	710	VDFTVDTNGVYSEPRPIGTRYLTRNL	736
AAV5	699	VDFAPDSTGEYRTTRPIGTRYLTRPL	724
AAV6	711	VDFTVDNNGLYTEPRPIGTRYLTRPL	736
AAV7	712	VDFAVDSQGVYSEPRPIGTRYLTRNL	737
AAV9	711	VEFAVNTEGVYSEPRPIGTRYLTRNL	736
AAVrh10	713	VDFAVNTDGTYSRPIGTRYLTRNL	739

**Supplementary Figure S4: Comparison of AAV-B1 capsid protein sequence to AAV8 and other natural AAV isolates.** The top two lines highlight the similarities and differences between amino acid sequences of AAV8 and AAV-B1, with singleton residue variants relative to AAV8 highlighted in red. The residues highlighted in pink indicate variant residues with no corresponding orthologs in the nine AAV species presented in the alignment. Translation start sites for VP1, VP2, and VP3 are indicated with filled triangles. The conserved parvovirus phospholipase A2 domain (approximately residues 44 to 104) in the VP1 unique region with the conserved AAV calcium-binding motif (Y-X-G-P-G/F) and catalytic residues (H-D-X-X-Y) are indicated with filled rectangles. The secondary structural elements are labeled with the corresponding text and the following symbols:  $\beta$  sheets B, C, D, E, F, G, and I are indicated with a horizontal overlined arrow. The positions of the variable loops (VR), I through IX, are indicated. The position of the conserved  $\alpha$  helix is indicated with three parallel horizontal lines.



**Supplementary Figure S5: Biodistribution profile of AAV-B1 and AAV8 vectors infused systemically at  $5 \times 10^{11}$  vg.** AAV vector genome content (N=4 animals per group) in **a.** CNS, **b.** liver and **c.** skeletal muscle (quadriceps) is shown. \*p<0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001 by Student's unpaired two-tailed t-test.



**Supplementary Figure S6: Antibody resistance assay comparing AAV9 and AAV-B1.** AAV9 or AAV-B1 vectors encoding GFP were incubated with control media or media containing the indicated concentrations of intravenous immunoglobulin (IVIg) that contain anti-AAV antibodies. Mixtures were added to HeLa cells and 48h later, cells were subjected to flow cytometry to determine the percentage of cells expressing GFP as well as the brightness of fluorescent signal (mean fluorescence intensity, MFI).